SB
608
T9
T61

Cornell University Library
SB 608.T9T61

Tomato anthracnoses,

3 1924 002 888 620

mann

TOMATO ANTHRACNOSES

BY

WENDELL HOLMES TISDALE

A Thesis Submitted for the Degree of

MASTER OF SCIENCE

UNIVERSITY OF WISCONSIN

1915

Cornell University Library

The original of this book is in the Cornell University Library.

There are no known copyright restrictions in the United States on the use of the text.

http://www.archive.org/details/cu31924002888620

CONTENTS

- 1^1 INTRODUCTION
- 1^2 GLOEOSPORIUM COMPARED WITH COLLETOTRICHUM
  - 2^1 Distribution and economic importance of the disease
  - 2^2 Symptoms
  - 2^3 History of the disease and its cause
  - 2^4 The causal organism, Gloeosporium phomoides Sacc.
    - 3^1 Description of the fungus
    - 3^2 Isolation of the fungus
    - 3^3 Cultural characteristics
    - 3^4 Temperature relations to spore germination and growth
    - 3^5 Physiology in liquid medium
    - 3^6 Results of inoculation, and mode of penetration
    - 3^7 Relation of fungus to host
    - 3^8 Dissemination
  - 2^5 Control measures
- 1^3 MELANCONIUM SPECIES
  - 2^1 Occurrence and economic importance
  - 2^2 Symptoms
  - 2^3 The causal organism, Melanconium sp.
    - 3^1 Description
    - 3^2 Comparison with literature
    - 3^3 Isolation and cultural characteristics

~~DEPT. PLANT PATH. CORNELL UNIVERSITY~~

3^4 Inoculation experiments

3^5 Relation of fungus to host

3^6 White flies as carriers

2^4 Possible control measures

1^4 SUMMARY

1^5 LITERATURE CITED

TOMATO ANTHRACNOSES

INTRODUCTION

The anthracnose diseases of the tomato are confined largely to the fruit, and are commonly known as "ripe rot" or "fruit rot". Rolfs (15) reported the disease caused by Colletotrichum lycopersici as producing a leaf and stem spot. His conclusions as to the cause of the leaf and stem spot were, no doubt, too hasty, as this is the only case in which leaf and stem injury of the tomato have been reported as being due to either of the anthracnose producing organisms. The writer has found in his investigations that one of these organisms, Gloeosporium phomoides will attack young seedlings just at and after the germination stage, but he has never been able to produce the disease on older plants. The most serious injury, however, is the decay of the ripe fruit.

The term "anthracnose" is a term applied to the disease and should not bear any relation to the causal organism. There is, however, a tendency to associate the term with certain conidial forms of the family Melanconiaceae, especially Gloeosporium and Colletotrichum. The term has been restricted to some extent to the disease caused by members of this family and closely related forms; still there is no reason why it should not be applied to any disease characterized by a perceptible shrinkage of tissue.

The cause of tomato anthracnose has been attributed by various investigators to two different organisms, namely, Gloeosporium phomoides Sacc. and Colletotrichum lycopersici Ches. In 1893 Chester (4) concluded that there was only one

organism causing the disease and that it was a Colletotrichum. p.2 He retained the old species name of Saccardo and called the fungus Colletotrichum phomoides Sacc. The work of later investigators (14, 17 and 18) still indicate that there are two organisms causing the anthracnose disease of tomatoes as was mentioned above. Practically the only difference between the two organisms is the production of setae by Colletotrichum. Chester (4) claims that with age and proper conditions the Gloeosporium will produce setae and become a Colletotrichum. Stoneman (18) and Pool (14) say that setae are not always present on the Colletotrichum. These conditions probably led Chester to conclude as he did. Stoneman (17 and 18) published on both Gloeosporium phomoides and Colletotrichum lycopersici. The organism with which the writer has been working is clearly a Gloeosporium. His work seems to confirm the conclusions of Stoneman, that there are two organisms causing the disease. In addition to these he has found a species of Melanconium which produces an anthracnose of tomato fruits.

The writer has had opportunity to study only Gloeosporium and Melanconium and will confine most of his discussion to these two. In order to clear up the distinction between Gloeosporium and Colletotrichum a discussion of the important literature dealing with the causal organism of this type of anthracnose will be taken up in this paper under the subject, Gloeosporium phomoides, etc.

In addition to discussing the literature, the purpose

of this article is to give a complete record of the advanced work done on Gloeosporium and to record the work and observations up to the present on Melanconium. The additional work on p.3 Gloeosporium is concerned largely with a study of cultural characteristics, temperature relations to spore germination and growth, nutrition, mode of infection, relation of parasite to host, and the relation of host seed to dissemination. The discussion of Melanconium will be confined, for the most part, to the description, infection, and possible means of control. Other factors will, however, be given some consideration.

GLOEOSPORIUM COMPARED WITH COLLETOTRICHUM

1. Distribution and economic importance of the disease

The disease is widely distributed throughout the United States. It has been reported in Massachusetts, New York, New Jersey, Delaware, Indiana, Louisiana, and Florida. Reports have also come from Italy, France and New South Wales. In most cases the disease has been considered of minor importance economically. Halsted (13) claims that the disease is of minor importance in New Jersey, not being as common as black mold. Rolfs (15) reports considerable damage to tomatoes and eggplants in Florida in 1905. This damage was, no doubt, not all due to this disease as he reports considerable damage to leaves and stems. Edgerton (8) says that the disease is of very little importance in Louisiana. Diseased fruits were obtained from Ralph Kemp, Tipton, Indiana, who makes the following statement: "As this disease in increasing in economic importance in this section, we will probably be forced to use control measures in the future".

It is possible that the disease will become more destructive in other sections where weather conditions are suitable.

2. Symptoms

Tomato fruits with anthracnose produced by Gloeosporium
p.4 phomoides are characterized by depressed or sunken areas which
vary in size from very small to one or more centimeters in diameter and in some cases involve almost the entire fruit. There may be one or more of these areas on a fruit and they may become confluent, which is usually true when the larger part of a fruit is involved. The depressed areas are more or less circular, spreading from a common center of infection. They vary in color from light or yellowish-brown to almost black, becoming darker with age. In the earlier stages the central portion of the spot is somewhat lighter in color than the surrounding area. Beneath this light colored area is a dry mass of white mycelium of the fungus. At this state of development small yellowish spots can be seen beneath the epidermis of the diseased area about midway between the center and margin of the infection. These spots gradually become darker and push outward, producing the black, erumpent fruiting bodies or acervuli. Some of these fruiting bodies break through the epidermis, causing a roughening of the surface, and producing spores which may show a slight pinkish tinge when in mass. Some of the fruiting bodies remain beneath the epidermis of the fruit and sometimes deeper in the tissues. The acervuli, as a rule, have no definite arrangement, although they may, in cases, be so arranged as to give indications of concentric rings. In the

later stages cracking of the disease areas opens the way for secondary infection by saprophytic organisms which cause a rapid decay of the fruit. The disease spreads faster in most cases from inoculations made by puncture than from natural infections. In the former case there is not so much shrinkage p.5 of tissue but more of a water soaked appearance of the affected area.

When carried on the seed the fungus will attack the young seedlings just as they emerge from the seeds, causing a darkening and decay of the seedlings very much like the ordinary damping off fungi. The leaves of the young seedlings show dark brown spots before the seedlings completely succumb to the disease. Blossoms of the tomato, when infected artificially, wither, the pistil darkens, and they decay.

Stoneman (17) describes the anthracnose of tomato fruits caused by Gloeosporium phomoides as follows: "An anthracnose causing a ripe rot of the tomato is manifested at first by a small, circular, depressed area. Older spots show a lighter central portion surrounded by a dark marginal band 2 to 3 cm. in diameter. Upon the central portion the dark colored fruiting pustules appear, producing irregular fissures in the epidermis, which often turn a light yellow on the margins. From the pustules the conidia ooze out in light pink masses. With age the diseased portions become quite black".

Chester (2) who was the first investigator to attribute the disease to a Colletotrichum describes the symptoms as

follows: "The disease shows itself upon the tomato as sunken, discolored spots, each with a dark center, becoming black. These spots increase in size or by a confluence cover a large portion of the decaying fruit. Over this area the fruit is black and sunken, flattened or depressed, surrounded by shrunken, corrugated, discolored skin". This description of the symptoms of Colletotrichum differs considerably from
p.6 the symptoms of Gloeosporium as given by Stoneman.

Pool (14) gave the following description of the symptoms of *Colletotrichum lycopersici* Chester: "The diseased spots may vary from small sunken, smooth areas, sometimes darker in color than the surrounding healthy tissue, again yellow and dotted with the small fruit masses or acervuli of the fungus, to large roughened areas, brown to almost black, covering an entire side of the fruit. A great many of the older spots have a characteristic which is very noticeable, that is the zonation rings. In such a concentrically arranged spot the acervuli will be massed together in circles, while a portion of firmer tissue with no external evidence of disease intervenes. Fruits in the light developed the rings while those in the dark did not. The acervuli are found only after the growth has continued for some time."

For the anthracnose caused by *Colletotrichum lycopersici* Rolfs (15) gives the follwwing as symptoms: "The disease appears as small brown spots on the stems and leaves. Under favorable conditions these spots enlarge and the greater portion of the leaves may become involved. On the fruit it

forms sunken, discolored spots each with a dark center. As the spots increase in size the area is blackened and depressed, surrounded by sunken and depressed skin". This description of the fruit spot seems to be fairly characteristic of the disease as described by other investigators, but the stem and leaf spot have in no other case been reported as being caused by this organism and were, no doubt, caused by an entirely different organism.

There seems to be a difference, as given in literature, between the symptoms of tomato anthracnose
p.7 caused by Gloeosporium and that caused by Colletotrichum. Stoneman (17) makes no mention of zonation rings formed by the acervuli of Gloeosporium, as described by Pool (14) for Colletotrichum. In the author's own observations of Gloeosporium no visible zonation occurred on fruits that could be considered characteristic.

3. History of the disease and its cause

The literature relating to the disease and its cause has been appearing quite often for a number of years, and is fairly well divided between Gloeosporium and Colletotrichum.

Saccardo (16) described Gloeosporium phomoides as producing ripe rot of tomatoes in Italy in 1878.

In 1884 Arthur (1) reported a fungus as causing ripe rot of tomatoes in New York. The fungus, he claims, develops just beneath the epidermis or skin of the fruit, and soon breaks through it and produces great numbers of spores on the ends of the protruding mycelium. He compared the

organism to *Gloeosporium phomoides* Sacc.

In 1891 Chester (2) reported the occurrence of tomato anthracnose in Delaware. He found the organism to be a Colletotrichum and gave it the name *Colletotrichum lycopersici*, believing it to be a new species. In 1892 he (3) says, "I was particularly struck by its entire absence, but in its place and equally common was another form of the disease. The fungus which in 1891 was clearly a Colletotrichum from the abundant development of setae was in 1892 without setae, and was to all appearance a Gloeosporium. In comparison of the two forms, leaving out the question of absence of setae, no definable difference can be stated. The spore measurements p.8 vary within the same limits, and other characters are quite identical". In 1893 he (4) says, "In the case of tomatoes artificially infected in the laboratory with spores of *Gloeosporium phomoides*, a good growth resulted, consisting at first only of conidia, a pinkish stroma without setae, but which afterwards turned darker and developed perfect setae. From this I have no further reason to doubt but that the *Colletotrichum lycopersici* of 1891 is identical with the *Gloeosporium phomoides* of Saccardo, in which case the new name becomes *Colletotrichum phomoides* Sacc."

Cobb (5) described a "pimply rot" of the tomato in New South Wales in 1895. The symptoms as given by Cobb and his plate showing the diseased fruit agree with Colletotrichum fairly well, but his spore measurements and drawings do not agree. His spore outlines illustrate very well the oil droplets

which are so abundant in the mycelium and perithecial bodies of *Gloeosporium phomoides*.

Halsted (11 and 12) reported *Gloeosporium phomoides* as causing ripe rot of tomatoes in New Jersey in 1895 and 1896. In 1903 he (13)again reported the disease and attributed the cause to *Colletotrichum phomoides*.

Stoneman (17 and 18) in 1898 concluded that there were two different organisms causing anthracnose of tomatoes, and described them separately as *Gloeosporium phomoides* Sacc. and *Colletotrichum lycopersici* Ches. She makes a distinction between the symptoms of the disease produced by the organisms and also between their colony characteristics in culture. *Gloeosporium phomoides*, she says, "forms a well developed cup-shaped stroma, lying some distance beneath the epidermis p.9 from which the short continuous basidia arise. They do not project beyond the host, but the conidia are delimited beneath the epidermis. The acervuli seldom become confluent. The acervuli in the case of *Colletotrichum lycopersici* were more erumpent, rusty brown or black and produced black perithecial bodies which were sterile. Setae were either present or absent".

Gueguen (10) in 1902 reported the occurrence of both Gloeosporium and Colletotrichum on the tomato in France. He says that *Gloeosporium phomoides* seems to be identical with *Sphaeronema lycopersici*, as it is a pycnidial form. His work indicates that the Gloeosporium causing the tomato anthracnose does not belong to the Glomerella type of anthracnoses. He seems

to have mistaken the perithecial bodies for pycnidia.

Rolfs (15) in 1905 says that Colletotrichum lycopersici caused fruit rot of tomato and egg plant in Florida. This is the only case reported of the fungus occurring on a host other than the tomato. He also reported serious stem and leaf injury to the tomato.

Edgerton (7) in 1908 and Edgerton and Moreland (8) in 1913 report the occurrence of tomato anthracnose in Louisiana and attribute its cause to Gloeosporium fructigenum, believing it to be identical with the Gloeosporium of the apple.

In 1908 Pool (14) did considerable work with tomato anthracnose caused by Colletotrichum lycopersici. She agrees with Stoneman in that there are two different organisms causing the disease. She says, "Confusion seems to have existed as to the proper naming of the anthracnoses. However, conclusions drawn from these previous investigations p.10 point to the occurrence of two anthracnoses upon the tomato, one produced by Gloeosporium phomoides Sacc. and one by Colletotrichum lycopersici Ches. The former is characterized by an imbedded acervulus without setae, while the latter forms an erumpent acervulus with or without setae".

The latest report of the disease available was by Ralph Kemp of Indiana in Oct. 1914. Specimens of diseased fruits were obtained from Kemp for investigation. The causal organism has proved to be a Gloeosporium. This is a further indication that the Gloeosporium causing tomato anthracnose

is distinct from the Colletotrichum causing a similar disease.

4. The causal organism, Gloeosporium phomoides Sacc.

Description of the fungus

The fungus is characherized by a branching, closely septate, colorless mycelium which contains an abundance of oil droplets. There are two general types of mycelial fibers, one which is rather uniform in diameter, contains few oil droplets and is considerably smaller than the other type which is irregular in diameter, contains numerous oil droplets, and is more closely septate than the smaller type. Conidia are borne singly by the pinching off of the ends of the fruiting hyphae which are massed together in an acervulus. These fruiting hyphae are short and erect, arising from a well developed basal stroma. Conidia are produced indiscriminately throughout the diseased tissues in the early stages, the acervuli being produced later. Conidia from diseased fruits measure 3 to 5 x 8 to 18 microns, the average being 4 x 12.3 microns. They are more commonly oblong with a slight constriction in the middle and contain
p.11 two vacuoles, one at each end. They are hyaline, slightly pointed at the proximal end, giving the spore a somewhat clavate form. Under favorable conditions the mycelium grows in compact masses which become black in from one to three days after they begin to form. The cells of the mycelium within these bodies round up and their walls become thick and black. These blackened bodies are filled with oil

droplets. When the individual cells of the mycelium begin to round up there is a rapid concentration of oil droplets within them. These bodies very closely resemble perithecia in outward appearance but have never been found to produce asci or spores of any kind. At high temperatures and in liquid media chlamydospores are produced in abundance by the rounding up and thickening of the walls of the mycelial cells. These chlamydospores have a greater diameter than the mycelium from which they were produced.

The original description of Gloeosporium phomoides by Saccardo (16) is as follows: "Conidia oblong, clavate, abruptly attenuate, apices rounded, 2.5 to 3 x 10 to 12µ, biguttulate, hyaline, basidia fasciculate, 1.5 x 20 to 21µ, hyaline.

Stoneman (17) says: "The spores may be as large as 5 to 6 x 18.5µ. In shape they may be oblong, elliptical, fusoid or reinform; and sometimes curved. The conidia germinate readily, frequently becoming once septate. The colonies show the dense, elongated y-shaped, or stellate center. With age the roseate tinge of the colony is less marked. The mycelium is nearly or quite white until a stroma begins to develop. This usually makes it appearance in a circle about mid-way between the center and the margin, when the colony is from six to nine days old."

p.12 Since Gloeosporium and Colletotrichum of the tomato are so nearly alike in general, it will probably be better to give the description of Colletotrichum lycopersici from

literature as this will help to clear up the distinction between the two.

Chester (2) gave the following description of Colletotrichum lycopersici: "The fruiting bodies consist of numerous spore bearing threads (basidia) with which are associated long bristle like organs (setae). The basidia are at first grouped in erumpent tufts (acervuli) arising beneath the cuticle and rupturing the same, but by confluence these distinct acervuli become obliterated and a uniform stroma covers the black center. The spores are borne upon and produced by the constrictions of the basidia.

The acervuli are abundant, densely gregarious, rusty brown to black, applanate, 95 to 150μ in diameter. Setae abundant, fuliginous, generally curved, rarely undulate or straight, often geniculate, in places gradually tapering, septate, length 65 to 112μ. Spores oblong, 16 to 22 x 4μ, averaging 18 to 20 x 4μ. Ends sub-acute, hyaline, generally containing two or three oil droplets which stain brown with osmic acid. Basidia short, slender, 30 to 40μ, arising from a well developed stroma."

Stoneman (18) adds the following to Chester's description: "There is a scant development of decumbent, spreading mycelium, with a strong tendency to concentric markings in the growth, where the mycelium is more erect and in tufts, surrounding black, spherical perithecia-like bodies which produce long setae. These, so far as has yet been determined, are sterile. The conidia formed freely

p.13

on the mycelium do not mass up in large heaps. Toward the margin, clusters of knotted and swollen mycelium are formed bearing quantities of dark colored buds or gemunae. These are close together but are more or less distinct".

Pool (14) gives the following description for Colletotrichum lycopersici: "The conidia measure 10 to 18 x 2.5 to 5.5µ which is somewhat less than that given in the original description. Under natural conditions, an examination of the tissue of an anthracnose spot which has not yet produced acervuli shows an abundant production of primary spores, which are formed in the same manner as that of the conidia in culture. As the spots become older, pink acervuli may be formed. These invariably consist of parallel arranged fungus tissue whose hyphal ends bear the setae, and surrounding spores, which are identical in size and appearance with the primary spores. As these acervuli become older, the central portion of the fungus tissue turns black. This continues until the entire acervulus is one blackened heap. The setae apparently break or fall off, as little or no evidence was found of their previous existence. The conidia also disappear, falling upon the surrounding tissue or scattering elsewhere. The spherical, dark aggregations are identical with those produced in artificial culture. They germinate readily, producing a vigorous growth when placed under favorable conditions.

The appressoria as found in this species are oval bodies, smooth in outline or with knob-like projections,

p.14 dark brown and contain a few large oil drops. They are invariably the first objects to come into focus when a hanging drop preparation is examined. Certain hyphae seem to grow up toward the coverglass and when it is reached, through mechanical stimulus or otherwise, the attachment organs are formed".

Isolation of the fungus

Two isolation methods were used, these being the more common methods of spore dilution and fragment cultures. Spore dilutions were made in tubes of sterile water and a few drops were placed on sterile potato agar poured in petri dishes. Diseased fruits were sterilized on the surface and fragments cut from the diseased tissue were placed in dishes of potato agar. As soon as growth began transfers were made to tubes of agar.

Cultural characteristics

The fungus presents different characters in color, growth, and fruiting on the different culture media. These variations are, no doubt, due to the different nutrient elements and the difference in proportion and combination of these elements in the various media. Some of the media contain the more important elements in a readily available form, so that the fungus grows very vigorously, while other media seem to have less of the necessary available food materials. The difference in consistency of the media may determine, to some extent, the characters of the fungus. A number of the more commonly used media were employed for

the growth of this fungus, and the following are the most noticeable characters on each medium:-

On tomato agar. Tubes cultures showed a white mycelial growth one day after inoculation. On the second day the central portion of the growth was slightly pinkish and conidia were p.15 being produced. Four days after inoculation, black perithecial bodies were present and the mycelium was beginning to disappear. After three weeks there were no visible signs of mycelium, only the black perithecial bodies remained.

On plates of tomato agar the growth was very adherent to the medium, and in cases showed very characteristic rings of growth. Few perithecia were produced, these being in somewhat concentric rings. The mycelium remained almost white, with a slight tinge of smoky-gray or pink. In both cases a 2 per cent agar in strong tomato decoction was used.

On oat agar (+5) growth was rapid. In four days it appeared deep pink in color with white border, and conidia were produced in abundance. After six days perithecia appeared. These black perithecial bodies were larger and more superficial than those porduced on tomato agar. The most rapid growth of the fungus was on this medium.

On (-30) beef broth agar growth continued slowly for more than a month when a few small, brown perithecial bodies were produced. The growth adhered very smoothly to the medium. The mycelium was at first white, but later turned a yellowish brown.

On slants of potato agar (2 per cent) the mycelial

growth was about the same as on tomato agar, but there was considerably less perithecial formation. The color was whiter than the color of the mycelium on tomato agar.

There was a very rapid growth of pinkish mycelium on cooked, sterile green bean pods four days after inoculation. p.16 The mycelial growth was very superficial with a white margin. Perithecia were being produced. After twelve days, the entire pods were covered with black perithecial bodies. On examination an abundance of conidia and chlamydospores were found. The perithecial bodies later became confluent, forming a black crust-like mass. A white mycelial growth appeared above this mass giving it a frosty tinge.

On cooked tomato stems the characters were about the same as on cooked bean pods (green), except that there was not such an abundant perithecial development. The perithecia did not become confluent. Conidia were fewer than on bean pods.

Tube cultures on 2 per cent plain agar slants grew very slowly. The mycelium remained white and only a very few perithecial bodies were produced. Perithecial production began two days later than on tomato and oat agar.

On tube slants of synthetic agar (2 per cent) the fungus grew fairly rapidly. The mycelium was entirely superficial and at first white, but it assumed a pinkish or roseate tinge after five days of growth. After nine days the colonies were gray in the center with pink margins. The surface of the culture medium was becoming dark and crumpled.

Twenty-three days after inoculation the mycelium was piling up in the center of the colony and was of a dark smoky gray color in that area. The margins were still pink. Perithecial bodies were being produced, but were almost flat. They finally became confluent, producing a rough, black stromatic mass. After the perithecial bodies were beginning to form yellowish almost translucent droplets of conidia were extruded from the p.17 cultures in abundance. Two forms of Gloeosporium from the apple were carried in culture on this medium under the same conditions but presented quite different characteristics.

On corn meal agar (1.5 per cent) growth was vigorous. The mycelium had a fluffy appearance. It was a grayish pink in early stages but became grayish-green to a dirty green in eighteen days. The surface of the medium was also of a greenish-black at this time. Perithecial bodies were produced in abundance, and were more irregular and larger in size then those on tomato agar. The perithecial bodies on all media first appear as brownish or pinkish masses of mycelium.

No descriptions have been recorded in literature of the cultural characteristics of Gloeosporium, but considerable results are given for Colletotrichum, some of which it might be worth while to mention, since they are practically identical with Gloeosporium in many respects.

Pool (14) describes Colletotrichum as follows:- "Upon artificial culture media, young colonies are white to pink in color. Later the colony commences to turn dark

at and around the central or older portion. The cells enlarge two or three times their usual diameter, become more or less spherical in shape, form many vacuoles, and take on a dark brown color. Few, if any, conidia are found in this darkened portion, although they occur abundantly near the edge of the colony where the new growth continues. As the darkened masses are developed, the conidial formation ceases. The spores, which have been produced, germinate and grow into a new mycelium. The latter, due to the lack of
p.18 nutrition, does not produce spores a second time. The blackened areas heap up until they are more or less spherical and somewhat confluent. They are sterile and may or may not produce setae. At times, especially in freshly isolated cultures pinkish heaps precede the formation of the black aggregations. They seem to be the typical acervuli with setae and spores".

Pool (14) gave the following descriptions of Colletotrichum on individual media:

"Tomato agar. Cultures one week old. Growth heavy, compact, membrane like, pink, with an abundant production of spores. Later, growth roughened with the black masses of acervuli scattered over the surface.

Stewed tomato. At first, mycelial growth clinging to the surface of medium, brownish, pink. Later, surface blackened with masses of acervuli.

Three per cent glucose agar (+10). The fungus presents the same appearance as upon tomato agar.

Corn meal. At first mycelium grayish green, not membranous, but rising slightly above the medium; colony edges pink, shading into white. Later when mycelial growth is carefully scraped away the characteristic small heaps or acervuli may be seen next to the medium.

Potato. Mycelium pink, edged with white and covering medium closely. Acervuli next medium hidden by the filamentous growth.

Carrot. Growth resembling that upon the potato.

Banana. Mycelium at first fluffy, later, in month old culture, medium shrunken, black, covered with a roughened heap of acervuli".

p.19 Stoneman (18) described the characters of Colletotrichum on bean stems as follows: "Very little mycelium is developed, but the stems are plentifully covered with black spherical or hemispherical pustules which bear long setae. In some of these bodies setae are absent. The bodies are sterile".

These descriptions as givem by Pool and Stoneman fit the characteristics of Gloeosporium fairly well, leaving out of consideration the development of setae which is entirely absent in Gloeosporium. The writer found quite often in old cultures that the conidia had largely disappeared. This was, no doubt, due to their germinating as Pool says. This was especially true in liquid media (to be given later) where the spores were found germinating. Germinating spores were also found in cultures on synthetic

agar.

Temperature relations to spore germination and growth

The object of this series of experiments was to determine the range of temperature within which spore germination and growth were possible, and to determine the optimum temperature for the growth of the organism. The series of temperatures was not altogether satisfactory for best results, but did fairly well for comparative results.

Hanging drops were prepared from spore suspensions in tap water and placed at the temperature given in the following table. The cells were placed on slides and duplicates placed in petri dishes containing enough water to prevent drying out of the drops. The percentage of germination was estimated in each case by counting the p.20 germinated and ungerminated spores in a number of fields of vision under the microspope, and then figuring the percentage of each from this data.

Table 1 gives the results of spore germination in tap water.

Table 1

No.of dish	Time of incubation	Temperature	Results
1	48 hrs.	2° C.	No germination
2	" "	3° "	" "
3	" "	5° "	15% "
4	" "	7° "	60% "
5	" "	11° "	80% "
6	" "	12° "	80% "
7	" "	13-1/2°C.	85% "
8	" "	14-1/2° C.	90% "
9	" "	15° C.	90% "
10	" "	17° "	90% "
14	1-1/2"	21° "	5% "

A part of this spore suspension was placed in small vials and placed out in the snow to freeze. One of these vials was taken in and placed at 36°C. after two hours. At the end of 48 hours there was about 1 per cent germination. The other vial was left in the snow for four hours at which time it was frozen through. It was then placed at room temperature (21°C). At the end of 48 hours there was no germination. The writer is unable to explain this result, as he has since that time had spores germinate after being left at much lower temperatures and for
p.21 a longer time. However, the latter were on the surface of a solid medium. Perhaps the process of freezing injured the spores mechanically and prevented germination.

Spores germinate in tomato decoction about as readily as they do in tap water. Hanging drops were prepared in the same way as with tap water. In this case the germ tubes were measured, so far as possible, in order to get some idea of the relative rate of growth at the various temperatures. Table 2 will show the time of incubation, temperatures, percentage of germination, and the average length of the germ tubes in cases where it was possible to make such measurements.

Table 2

No.	Temperature	Time	% germination	Av.length of germ tubes
1	2° C.	3 days	0	0
2	4° "	" "	0	0
3	5° "	" "	1	3µ
4	8° "	" "	15	40µ
5	12° "	" "	25	40µ
6	14° "	" "	50	343µ
7	15° "	" "	80	Longer
8	16-1/2° "	" "	85	Not meas. still longer
9	19-1/2° "	" "	85+?	Bearing conidia
10	23° "	22-1/2 hrs.	90	394µ
11	27° "	22-3/4 "	90	692µ
12	30° "	28 "	48	Not measured
13	34° "	22-3/4 "	12	225µ

p.22 The above table shows in a relative way what temepratures are best for spore germination and growth. More accurate results might have been obtained had the time been the same for all measurements and percentage calculations. In numbers 7, 8, and 9 the germ tubes were too long and entangled to measure. In number 9 the percentage of germination as given is probably not accurate, as the mycelium was producing conidia at the time of measurements and there is a chance that some of the newly formed conidia were mistaken for old ungerminated ones. In the last four cultures of this series the time of incubation is very irregular, but is of some significance. This irregularity is due to lack of time on the part of the author to give the desired attention to the work. The results would have been of more value if higher temperatures had been available at the time of this experiment. The percentage of germination and growth was greatly decreased at 34° C. This indicates that the maximum temperature is not far above that point. This is actually the case as spores failed to germinate on agar at 36° C. The results of this table might be arranged in the form of a curve, or number of curves. A curve could be plotted for the per cent of germination at any one time for the various temperatures. This curve would reach the temperature axis at about 5° C. and 35° C., having its highest point between the points represented by 23 and 30°C. A curve of this nature could be plotted for the length of germ tubes. Another curve might be plotted for time of

germination at the various temperatures. Using one axis for temperatures and the other for hours, the curve would be a reverse of the others with its lowest point at the point of optimum temperature for germination. p.23

One of the main difficulties met with in these spore germination tests was the obtaining of uniform spore suspensions. When the spores are massed together the percentage of germination is very low at any temperature, the larger per cent of the germination being around the edge of the mass. There seems to be some tendency for the spores to come together in the hanging drop.

An experiment was arranged to determine the thermal death point of spores. A wire basket was fitted in a boiler, so that it was well away from the bottom and its corners were the only parts in contact with the boiler. Tubes of agar placed in this basket were kept from coming in contact with the metal of the boiler, which was filled with water and placed over the flame. Tubes of tomato agar were used. A thermometer was placed in the basket with the tubes. As soon as the desired temperature was reached the tubes were inoculated with spores and the flame regulated to keep the temperature constant. At the desired time the tubes were removed from the basket and placed at 27° C. Table 3 will show the results of duplicated experiments.

p.24 Table 3

No.	Time heated	Temperature	Results afterwards
1	10 min.	48°C.	Growth
2	" "	49 to 50°C.	Growth
3	" "	50 to 51°C.	No growth
4	" "	52 to 53°C.	" "
5	" "	53 to 54°C.	" "
6	15 "	54°C.	" "

Table 3 shows that the thermal death point of spores of this fungus is between 50 and 51°C. This experiment was repeated, using tubes of sterile water instead of agar. When the desired temperature was reached, a drop of spore suspension was placed in the tubes. After heating for the desired length of time the solution was poured over dishes of sterile tomato agar and incubated. Spores heated in water for 10 minutes at 50.5 to 51.5°C. failed to germinate. It was suggested to the writer that the experiments with agar were incorrect, as agar failed to heat up as fast as water. In order to test this one thermometer was placed in a tube of the agar so that the bulb would be immersed in the middle of the agar and the thermometer would not touch the tube at any place. This was done by pushing the thermometer through the middle of the cotton plug. Another tube was prepared in the same way except that the bulb of the thermometer was just touching the surface of the agar. A third thermometer was placed in the basket with the tubes as in the first experiment. The heat was applied
p.25 very slowly as in the experiment. The agar heated up somewhat slower than the surrounding water, there being from 1.5 to

2.5°C. difference when the water reached 51°C. The temperature of the central portion of the agar was the lowest, when the temperature of the water was held constant for four minutes all thermometers read the same. When cooling down, the agar cooled slower in proportion, thus making the results of the experiment relatively accurate.

The resistance of spores to cold was also tested. This was done by placing them on the surface of agar slants and then placing the tubes in the snow. The first set of tubes remained on the snow for 24 hours. During the night the temperature went to 4°F. The tubes were brought to room temperature and in two days the fungus was growing vigorously. The fungus fruited in the normal manner. Another set of tubes was inoculated as before and placed out on snow over night. They remained out 17 hours. In this case the temperature went to -18°F. After placing at room temperature growth proceeded in the normal way. These results indicate that the fungus is capable of surviving very low temperatures. Two of the Gloeosporiums of the apple were placed under the same conditions in this experiment and survived as did the tomato organism.

Further studies were made on the growth and fruiting of the fungus at different temperatures on a number of different media. For this experiment tomato agar, plain agar, oat agar, cooked tomato stems, and corn-meal agar were used. The difference in medium did not alter the effects of temperature on the organism to a noticeable degree. Table 4 shows the relation of temperature to mycelial growth and fruiting and perithecial

production. These cultures were run for 22 days, except numbers 15, 16, and 17 which were run for a shorter time, but long enough for good results.

Table 4

No.	Temperature	Growth	Perithecia
1	0-1°C.	0	0
2	1-2°"	0	0
3	3-4°"	+ slight	0
4	6-1/2-7°C.	+ 3	+
5	10-1/2-11°C.	+ 6	+ 3
6	11-1/2-12°"	+ 7	+ 4
7	12-1/2-13-1/2°C.	+ 8	+ 5
8	14-14-1/2°C.	+ 9	+ 6
9	15-16°C.	+10	+ 7
10	16-1/2-17°C.	+11	+ 8
11	20-1/2-21-1/2°C.	+15	+10
12	23°C.	+16	+11
13	27°"	+20	+15
14	30°"	+18	+12
15	34°"	+ 8	+ 4
16	37°"	0	0
17	40°"	0	0

In the above table, visible growth or perithecial production is represented by the sign +. The numerals occurring with the plus signs represent the relative amounts of growth and perithecial production. Close observations p.27 were made every two days through most of the period of incubation in order to determine these results. It was impossible to get anything more than relative results, and yet these serve our purpose fairly well in giving us some idea of the range of temperatures at which the organism will grow.

Thinking that there might be some possibility of producing fertile perithecia by varying the temperature,

the writer placed the cultures grown in the above experiment at room temperature, for twelve days. At the end of this time all of the cultures which had been at low temperatures were growing vigorously. They were again placed in their original places in the incubator and kept there for twenty-nine days, after which time they were placed at room temperature again. The perithecial bodies were examined from time to time but were found to be sterile in every case. The series of cultures on corn-meal agar were not treated in this manner, but were placed at room temperature for two days, then placed at 27°C. for five days. At the end of this time they were placed at 1°C. Examination showed that the perithecial bodies were sterile. The temperatures employed seemed to have no affect in producing fertile perithecia.

Two tube cultures which were producing perithecia and five germinating cultures just inoculated with spores were placed in an incubator at 28°C., and the temperature was gradually raised, through a period of six days, to 46°C. The cultures remained at a temperature between 44°C. and 46°C. for five days. They were then placed at room temperature and moistened with sterile prune decoction. One of the cultures which had perithecia formed put out new growth, but
p.28 none of the cultures which were just germinated at the time the experiment was begun showed signs of growth. Perhaps it is the thick walled, rounded cells of the mycelium in the perithecial bodies which serve to resist the high temperatures more than do the spores and young mycelium.

Physiology in liquid medium

This fungus grows vigorously in a liquid medium if the necessary elements are supplied, thus making it possible to determine the relative value of each of the elements to growth and fruiting. The results given are to be considered as only relative, since time did not permit the carrying out of the desired number of confirmatory tests. Moreover, the compounds, containing the different elements used, might not have been altogether satisfactory, although they were the best available under the present knowledge of plant physiology. Considerable work has been done with the mold fungi with quite noticeable results. It is possible, however, that some of the investigators used more refined methods than were used in this work.

The necessary elements

A standard nutrient solution was used as a basis for the experiment. This standard solution was made up from a number of stock solutions which were prepared by dissolving each reagent in its proportional amount of doubly distilled water. These stock solutions were made up in the following proportions and of the reagents named:-

1.0 gr. KNO_3 in 20 c.c. distilled H_2O
.5 " KH_2PO_4 " " " " "
.25 " $MgSO_4$ " " " " "
p.29 2.0 mg. $FeCL_3$ " " " " "
5.0 " cane sugar in 20 c.c. " "

The above solutions contain the following elements:

C, H, N, O, S, P, K, Mg, Fe, and Cl. The sugar solution was not prepared until ready for use.

The importance of any one element was determined by omitting it from the solution and substituting for it a non-essential element. The substitutions were as follows:

Minus nitrogen:

For KNO_3 substitute KCl - 1 gr. in 20 cubic centimeters distilled H_2O.

Minus potassium:

For KNO_3 substitute $NaNO_3$ - 1 gr. ditto.

For KH_2PO_4 " NaH_2PO_4 - 1/2 gr. ditto.

Minus phosphorous:

For KH_2PO_4 substitute K_2SO_4 - 1/2 gr. ditto.

Minus magnesium:

For $MgSO_4$ substitute $NaSO_4$ - 1/4 gr. ditto.

Minus sulphur:

For $MgSO_4$ substitute $MgCL_2$ - 1/4 gr. ditto.

Minus iron:

For $FeCL_3$ substitute $NaCL$ - 2 mg. ditto.

Minus carbon:

For carbon nothing was substituted, only distilled water added.

A series of cultures were set up in duplicate, using 125 cubic centimeter flasks with 50 cubic centimeters of solution in each flask. Solutions were sterilized before
p.30 inoculating. The flasks were inoculated with equal volumes of a uniform spore suspension and allowed to incubate at

room temperature in the dark for two weeks. Notes were taken every few days on growth and fruiting. At the end of two weeks dry weights of the fungus were determined. Table 5 shows the dry weights of the different cultures. It will also show the element or elements left out of each culture.

Table 5

No.	Minus element	Weight of A	Weight of B
1	All elements but H_2O	0	0
2	All present (check)	.723 gr.	.819 gr.
3	Nitrogen	.192 "	.185 "
4	Potassium	.305 "	.234 "
5	Phosphorus	.212 "	Lost "
6	Magnesium	.529 "	.453 "
7	Sulphur	.283 "	.244 "
8	Iron	.603 "	.581 "
9	Carbon	0	0
10	All mineral elements	Very slight	Very slight

The above table shows that each element of the standard nutrient solution is more or less important as the dry weights in a full nutrient solution are the greatest in the table. The dry weights correspond very closely with notes on observations of growth. The table does not show the amount of fruiting in the cultures. The heaviest pro- p.31 duction of conidia was in the full nutrient solution. In cultures without nitrogen and phosphorus there was no fruiting. Where magnesium was absent perithecial bodies were produced in abundance, but these were sterile as usual. The absence of potassium, sulphur, and iron did not materially affect the fruiting as far as it was possible to observe. It was somewhat less than in the full nutrient solution.

Large numbers of chlamydospores were produced in all cultures where growth was appreciable.

Toxicity of copper sulphate in the presence of organic compounds

Three sets of cultures were set up containing carbon supplied as, (a) 4 grams corn starch, (b) 4 grams sucrose, and (c) 4 grams peptone per 100 cubic centimeters. Copper sulphate was added to all three series alike. The following strengths were used: m/250; m/500; m/1000; m/2000; m/4000 and m/8000. After sterilization, these solutions were inoculated as in the previous experiment and incubated at room temperature in the dark for three weeks. In the solutions containing peptone, $CuSO_4$ was not toxic with the strengths used. In solution with carbon supplied in the form of sucrose there was very little growth in m/1000 $CuSO_4$ and no growth in m/250 and m/500. With carbon supplied as corn starch, m/500 $CuSO_4$ was slightly toxic, and m/250 prevented a visible growth for two weeks. These results show $CuSO_4$ to be more toxic in sucrose solutions than in corn starch solutions, and more toxic in the presence of corn starch than in the presence of peptone. In another series of cultures with $CuSO_4$ the writer found weaker solutions, m/16000 and
p.32 m/32000, to be slightly stimulating to mycelial growth and more so to conidial and perithecial production. These cultures were prepared with the ordinary full nutrient solution with the addition of the $CuSO_4$.

Acidity and alkalinity

For this experiment the nutrient solution was neutralized to phenolphthalein, placed in flasks and sterilized. After sterilization n/1 HCl or n/1 NaOH was added to make the solution acid or alkaline as was desired. The acidity and alkalinity was adjusted to Fuller's scale. Care was taken to add the acid and alkali under aseptic conditions. This experiment was also carried out with oat-meal agar as a medium. After incubating at room temperature for ten days growth was very slight at both plus and minus 30 Fuller's scale. The range of growth was found to lie between +40 and -40 Fuller's scale. The growth at -30 was slightly better than at +30. Growth was best in a slightly acid medium (+5 to 10).

Influence of nutrition on secretion of enzyme

Most fungi are able to utilize a number of organic compounds as a source of carbon. The higher compounds such as starch must first be digested. The enzyme diastase has been found to accomplish the starch digestion. The fungus must secrete this enzyme in order to digest the starch.

To show the effect of different amounts of sucrose on the production of diastase a series of cultures were set up in which the sucrose in the standard nutrient solution was replaced by .25 grams of corn starch per 100 cubic centimeters of solution. The starch was dissolved thoroughly by
p.33 heating. Two series of flasks were prepared, A and B and sucrose added as follows:

To No. 1 of A and B - 0.0 gm.
" " 2 " " " " - 0.1 "
" " 3 " " " " - 1.0 "
" " 4 " " " " - 2.5 "
" " 5 " " " " - 5.0 "
" " 6 " " " " - 10.0 "

These solutions were sterilized and inoculated as previously mentioned. Series A was placed at room temperature and series B at 30°C. After ten days, portions of each solution were tested for starch with potassium iodide iodine solution. The starch was disappearing in the solutions where sugar was not added. It was being used faster at 30°C. than at room temperature. After twenty days the starch was entirely used up in 1 A, 1 and 2 B, and was decreasing in the cultures following these in the series. This shows that as long as the fungus can get its carbon in readily available form, as sugar, it does not secrete the enzyme diastase; but when the sugar disappears, and it becomes necessary to digest the starch in order to secure carbon, the enzyme is secreted. This fungus, however, needs very little carbon for growth as compared with Aspergillus niger. The time required for the latter to digest the starch was less than half the time required by Gloeosporium phomoides.

Results of inoculation and mode of penetration

Inoculations were first made on picked fruits in the laboratory. In each case the surface of the fruits was sterilized with mercuric chloride solution (1-1000) and washed with sterile water before inoculating. They were kept

in moist chambers at room temperature after inoculation. The following table will show the results of inoculations in the laboratory.

Table 6

Date	No. of fruits	Kind of fruit	Method of inoculation	Results
Oct.26	3	Ripe tomatoes	Needle prick	Typical infection on all fruits.
Nov.10	2	Apples	Needle prick	Brown, sunken areas on both fruits with black acervuli.
Nov.27	2	Green peppers	Needle prick	Perfect infection.
Dec.19	6	Ripe tomatoes	Needle prick	Perfect infection.
Dec.19	6	Ripe tomatoes	Spray	3 fruits infected.
Dec.29	8	Ripe tomatoes	Spray	4 fruits infected.
Dec.29	4	Ripe tomatoes	Needle prick	All inoculations successful.

The above table shows that when spores are introduced into the tissues of the fruits infection is almost certain under these special conditions. Inoculations by spray were not so successful. Microscopic examination showed that tomato fruits when kept in the moist chamber for several days had small cracks in the cuticle and epidermis. The infections on the sprayed fruits were not visible for a number of days, five days being the minimum. The fungus can easily get in after cracks occur in the epidermis. This is the case with ripe fruits kept under very moist conditions this length of time.

The larger part of the inoculation work was done
p.35 on fruits on the plants in the greenhouse. The writer feels that very little emphasis should be placed on results obtained from inoculations on picked fruits placed in a moist chamber. The fruits are far from being in their normal condition, and besides, almost any organism that will grow on culture media is

able to grow on a ripe tomato under such conditions. For this reason few inoculation experiments were carried on in the laboratory.

In order to bring out the results of the inoculations on plants more clearly, the results were arranged in the form of a table as given below (table 7). Inoculations were made on all parts of the plant, above ground namely, stems, leaves, blossoms, and fruit. In most cases where fruits were inoculated by spraying with a spore suspension, they were wrapped with moist cotton for one to two days to keep the surface moist until the spores had time to germinate. In this case results were no better than where the moist cotton was not used, as practically no results were obtained from spray inoculations. The temperature of the greenhouse was below the optimum temperature for growth of the organism and the moist cotton made the temperature around the fruit still lower.

p.36

Table 7

Date	Part of plant inoculated	No. inoculated	Method of inoculation	Results
Dec. 21	Stems	10	Needle prick	No infection
" "	Green fruits	10	" "	All infected
" "	Leaves	5	" "	No infection
Jan. 16	Green fruits	3	Spraying	" "
" "	Blossoms	5	"	3 infected
" "	Green fruits	6	Needle prick	All infected
Jan. 21	" "	3	Spraying	1 fruit infected
" "	Ripe fruits	2	Spraying	No infection
Feb. 1	" "	3	"	" "
" 6	" "	3	Needle prick	All infected
" 13	Green fruits	12	Spraying	No infection
" 25	" "	7	Slight needle prick	6 fruits infected
" 25	Blossoms	9	Spraying	5 infected
Mar. 15	Green fruits	15	Pricked and sprayed	All infected
" 18	Green and ripe fruits	14	Needle prick	" "

The above table points to the fact that the fungus is largely a wound parasite. This was further proved by the inoculations made by spraying a spore suspension on fruits which were very lightly pricked with a sharp pointed needle. Infection was obtained in each case but the fungus grew very slowly. The growth was more rapid in deeper wounds. In no case did the fungus attack leaves and stems of the tomato plant. Numerous inoculations were made by spraying a spore suspension on leaves and stems, but no infection was observed. p.37 The fungus is capable of infecting the blossoms when spores are sprayed in after the blossoms open.

The mode of penetration of the organism has been mentioned above to some extent. The main mode of entrance seems to be through wounds or some form of opening in the epidermis of the fruit. The tomato fruit has no lenticels in its epidermis, but has cracks in the cuticle which Groth (9] has described as follows: "All tomato skins contain single epidermal cells or patches of cells, usually smaller than the average, which appear brown and show distortion or abnormal thickening. They are caused by interference with the growth of cells. In almost all cases the cuticle and cuticular thickening above one or more of the cells were found cracked, and often germinating spores of molds were found in them. Where no spores entered, the wound seemed to have healed, but the cells had not expanded subsequently at the rate of the normal adjoining cells. In parts recently affected the contents of one or more cells were browned and

changed from the normal vacuolar structure into a brown mass containing oil globules and sometimes crystals not found in normal cells nearby. The radius of transformation of the cell contents extended at times over about ten cells. Hair bases from which the hairs are broken off are often browned in that way." In studying the tomato skins the writer found openings which fit the above description by Groth. Under favorable conditions, the fungus, no doubt, enters through these openings. Green fruits sterilized as mentioned above and placed in a moist chamber were sprayed with a spore suspension, covered p.38 with moist cotton and incubated at 27°C. In two days there was a russeting of the surface of the fruits. Microscopic examination showed that these very small brown areas were due to the browning of cells beneath minute rifts in the cuticle and in some cases in the epidermis. The writer was able to find the mycelium entering the tissue of the fruits through these openings. This slight russeting effect on tomato fruits is noticeable near the stem end of tomato fruits after warm muggy weather. The temperature and humidity which gives best conditions for this cracking of fruits is most favorable for the growth of the fungus. It is quite probable that a large percentage of infections in the field are brought about by these conditions. The ripening season for tomatoes is usually a season of high temperature and is quite often humid, depending to some extent on the location.

The fungus penetrates the flowers through the stigma and probably through the nectaries. The stigma turns black and

the infection gradually extends down into the ovaries. In case of young seedlings the fungus can enter without any wound at all. It seems to be able to penetrate any part of the seedling. To determine the latter, seeds from infected fruits were planted on moist, sterile filter paper in test tubes. The seeds germinated and started growth vigorously, but as soon as the fungus began to develop it attacked the young seedlings and caused a disintegration like the damping off fungi.

Relation of fungus to host.

The fungus is largely intercellular in new infections but becomes intracellular in the later stages. The invasion is p.39 not confined to the superficial parts, and may penetrate all parts of the fruit. Young fruits, about one half inch in diameter and less offer a resistance to the growth of the fungus when inoculated. Growth begins as vigorously as on larger fruits, but is checked in a few days. The wound seems to heal and the fruit grows to maturity. After ripening the fungus was able in most cases to begin growth again from the old spot and destroy the fruit. A study of sections through these spots where fungal growth was checked showed that there was a rapid cell division in the host tissue surrounding the infected area. There was a great increase in number of cells, the cells being much smaller than normal. Not only were the cells smaller and more numerous but the walls were thickened. This thickening was found to be suberin by testing with potassium hydroxide which gives a yellow color, and with saffranin which gives a

red color with suberin. Saffranin also gives a red color with lignin and cutin, but we should not expect to find these in the inner part of the tomato fruits. This stimulation to production of cork by the host tissue was a means of holding the fungus in check.

The cell content of the young fruit probably plays some part, or a large part, in checking the advance of the fungus in the tissues. It is hardly probable that the acid content plays any great part in these results as there is not a great difference in the acid content of green and ripe fruits. Furthermore, the higher acid content is in the ripe fruits. The writer found the juice of fruits half grown to be +50 and the juice of ripe fruits to be +80 Fuller's scale. These results correspond fairly well with those of Cook and p.40 Taubenhaus (6). Their analysis of the tomato fruit is as follows:

Table 8

Condition of fruit	Oxidizing power of enzyme	Acidity	Sugar
Green	5	.29	2.86
Green	5.6	.48	2.86
Mature	2.3	.58	2.77
Half ripe	.9	.78	2.91
Ripe	1.1	.56	3.02
Ripe*	.6	.58	2.05

*Kept a few days after picking.

This table shows that the oxidizing power of the enzymes decreases, while the acid increases, and the sugar remains almost constant while the fruit passes from the early

stages to maturity and ripening. Since the fungus grows best on a medium of very low acidity it is not likely that the increase in acidity enables the organism to grow on the older fruits while its growth is checked on very young fruits. There seems to be more correlation between the oxidizing power of the enzymes and growth than between the amount of acid, or sugar and growth. During the rapid growing period of the fruit when the oxidizing power of the enzymes is high, the tissue readily responds to stimuli by wound or invasion of fungi and, by rapid cell division and suberization of cell walls, is able to form a protection.

p.41

Dissemination

One method by which the fungus is disseminated is by means of spores and perhaps by bits of mycelium carried on seeds from diseased fruits. The author was able to show this by taking seeds from infected fruits, keeping them dry for over five months and then germinating the seeds on sterile, moist filter paper in test tubes. The fungus killed the young seedlings in each case (8) where the seeds were not treated to kill the fungus (see Plate II). Infected seeds when treated with bichloride of mercury (1-1000) for 1 to 5 minutes germinated and grew nicely. This is sufficient proof that the fungus is carried on the surface of the seeds. The spores may also be carried by wind, insects, water, and other agencies, although this has not been demonstrated. The spores are often superficial on infections and seem to be very easily rubbed off.

Control measures

Cobb (5) gives the following control measures for the "pimply rot" of the tomato which is perhaps the same disease:-

1. "Destroy affected tomatoes by fire. Such are worthless and only serve to spread the disease.

2. Avoid seed from all plants which have shown the disease.

3. Support the tomato vines on a high trellis work. Though this does not absolutely prevent disease it helps to keep the growth hardy, lets in more air and sunlight, and ensures a better crop.

If this disease has been severe on a piece of ground devoted to tomatoes, remove the tomatoes next season to ground as far distant as practicable, and preferably higher ground.

p.42 5. See that land is well drained.

6. It is barely possible that spraying with Bordeaux mixture once in ten days from the time of blossoming will prevent this disease."

Halsted (11) found Bordeaux mixture to be the most satisfactory spray for the control of the disease, yet it was very inefficient. The measures as given by Cobb would, no doubt, be fairly satisfactory.

The disease, being largely a disease of ripe fruits, would likely spread very rapidly if diseased fruits were introduced into shipments. The decaying fruits might drip and, thereby, spread the disease to other fruits near by. Fruits

packed for shipping would likely be wounded more than before picking and would be more easily infected. If the fruits are kept at a temperature of about 41°F. after picking there can be no chance for the fungus to grow as it fails to grow at a temperature this low. This can be accomplished by placing in cold storage until ready for use, and, when shipping by using refrigerator cars with a temperature somewhere near 41°F.

Since the fungus is carried on the surface and not in the interior of the seed, a seed treatment becomes important. Immerse the seed in a solution of mercuric chloride (1-1000) for a period of three to five minutes, remove and wash with water. This treatment is sufficient to kill all traces of the fungus and does not injure the seed. Further experiments on seed treatment are to be carried out later.

It might be well to mention the possibility of resistant varieties as a means of control. p.43 No experimental work has been done along this line up to this time, but it would be an important problem to consider.

MELANCONIUM SPECIES

Occurrence and economic importance

The only known instance of the occurrence of the disease was in the University horticultural greenhouse, Madison, Wisconsin. The disease was first observed in January, 1915. About 20% of the fruits in one section of the greenhouse were more or less infected. The disease occurs on fruits of all ages, and is more destructive than the Gloeosporium type of anthracnose when it enters through

wounds. Should this disease become widespread, it would likely be of considerable economic importance. It is, at least, capable of considerable destruction in the greenhouse.

Symptoms

The disease first becomes visible on green fruits as irregular brownish spots. Later they become darker in color and finally black. Surrounding the spots, in some cases, there are sunken areas, or slight indentations. The spots are very small, seldom reaching an eighth of an inch across. The skin of the ripe fruit may look a little yellowish around the spot but it usually has the normal color. The line of demarcation between normal and diseased tissue is sharp. The spots may be numerous on the fruits, occurring on fruits of all sizes, both green and ripe. The central portion of the spot is slightly raised above the surrounding sunken area in some instances. The diseased tissue is of a tough p.44 corky consistency. The spots which occur independent of wounds are confined to the superficial layers of the fruit.

The symptoms of the disease when produced by wound infection, are considerably different. Sunken areas are produced which are light to dark brown on mature and ripe fruits and black on young fruits. One spot may involve the entire side of a fruit. There is a distinct zonation in most cases, the zonation rings being of a darker color than the diseased area in general. After the spots are ten to twelve days old, yellowish specks or acervuli can be seen beneath the epidermis. These push through the epidermis and cuticle in the form of

an erumpent mass of whitish mycelium, which produces a black mass of spores. Spore masses are also produced in the carpel cavities of the fruit.

The causal organism, Melanconium sp.

Description

The mycelium of the fungus is white, branching, and septate. The diameter of the hyphae is fairly uniform, being about 3 microns. The conidiophores are short, unbranched, hyaline, closely compacted together in an upright form, producing an acervulus. They arise from a definite basal stroma and produce spores singly at the apex. Conidia are one-celled, cylindrical with rounded apices, light green when single and black in mass, 2 to 4 x 7 to 10 microns, the average being about 3 x 8 microns. They also transmit the light. Spores placed in hanging drops of tomato decoction at 23° and 27°C. germinated perfectly in a few hours.

p.45 The fact that the spores of the organism transmit light might cast some doubt on its being properly placed in the genus Melanconium. There seems to be no sharp line of distinction at this point. Since the spores of the fungus are colored and it agrees with the genus Melanconium more closely than with any other genus, in regard to other characters, the writer has placed it with the Melanconiums.

Comparison with literature

The species of Melanconiums described so far have been largely saprophytic. Saccardo described more than 100 species which were, for the most part, saprophytic on the bark of

forest trees. The writer has not been able to find any report of Melanconium on tomatoes. The fungus, however, fits the description of the genus as given by Saccardo, Stevens, Engler and Prantl, and others fairly well.

Isolation and cultural characteristics

The organism was isolated by the fragment culture method. The surface of the fruits were sterilized with mercuric chloride solution (1-1000) and washed in sterile water. The spots were then cut out under aseptic conditions and placed in sterile dishes of oat agar, and incubated at room temperature. The fungus is rather hard to isolate from the superficial type of spot.

The characters of the mycelial growth of this fungus are practically the same on all culture media used. The mycelium is superficial, white, and very profuse. On corn-meal agar, however, it becomes a greenish gray with age. The fungus fails to produce the black spore masses on potato agar. On tomato,
p.46 oat, and corn-meal agar the spore production is about the same.
The spores are produced in disk like masses which are very black and have a glistening, moist appearance. Very young spore-masses are a deep grass-green in color. The mycelium seemed to hold its uniform diameter on all media used.

Inoculation experiments

Inoculations with Melanconium were confined entirely to fruits on plants in the greenhouse. The methods used were, (a) spraying a spore suspension on the uninjured fruits and (b) inserting spores by needle puncture. Some of the fruits

were wrapped with moist cotton after spraying. The cotton was removed after one to two days. Results from inoculations where fruits were wrapped were better than where they were not wrapped. The results of inoculations with Melanconium are given in table 9.

Table 9

Date of inoculation	Fruits inoculated	Method of inoculation	Results
Jan. 16	5 green	Needle prick	All infected
" "	3 "	Spraying	No infection
" 21	3 mature	"	1 fruit infected
Feb. 6	7 green	Needle prick	All infected
" 13	12 "	Spraying	4 infected
Mar. 2	2 "	Pricked and sprayed	All infected
" 18 "	10 green to mature	Needle prick	All infected
" 27	9 green	Sprayed	4 infected. Stem of 1 fruit attacked
Apr. 5	12 green	Sprayed	1 infected

p.47 The above table shows that the fungus grows very readily on tomato fruits when introduced by wounds. It is also able to attack fruits which are apparently uninjured. In one case, as noted in the table, the stem of one fruit was attacked and destroyed to the extent that the fruit fell from the plant. After the fruit had fallen, perfect fruiting bodies of the fungus developed on the attacked parts. In every case where infection occurred from spraying, the fruits were wrapped with moist cotton after inoculation. The results obtained show the fungus to be a more destructive disease to the tomato than Gloeosporium.

Relation of fungus to host

Fruits of all ages are attacked. Young fruits are

not capable of checking the growth of the fungus when it is introduced by wounds, as was the case with Gloeosporium. J. C. Walker, while working with onion diseases in this laboratory, found a fungus which is morphologically identical with this fungus, but, when green tomatoes were inoculated with it, the invasion was checked in a few days by a cork layer which was laid down around the infected tissue. This shows that the fruit makes an effort to check invasion by cell division and cork formation, but, in the case of the tomato Melanconium, the invasion was too rapid to be held in check by this process. The mycelium within the tissue is largely intracellular. The small mycelial threads form a net-work within the cells of the fruits. The fungus is largely superficial, being confined to a few layers of cells when infections take place without wounds; but when infection is through a wound the entire fruit may be invaded.

p.48

White flies as carriers of the fungus

One noticeable feature in the greenhouse where the disease was found occurring was the abundance of white flies. It occurred to the author that these flies were probably carrying the spores of the fungus from plant to plant. There is no reason why this should not be the case as the acervuli break through the cuticle and the spore are borne in abundance and are very easily detached. During the experimental work the white flies got into the greenhouse. After they had been in the house for several days, infections of Melanconium, with its black spore masses on fruits which had been inoculated with

Gloeosporium were found. It is quite probable that the flies visited the fresh wounds for food after visiting fruits on other plants which had spores of Melanconium produced on them. The fact that the spores are borne in an exposed manner and in such large numbers would seem to make it possible for them to be disseminated in a large number of ways.

Possible control measures

1. A large part of the source of infection may be destroyed by sanitation. Pick and burn or bury all infected fruits.

2. In case the disease becomes important in the field rotation of crops would, no doubt, be of value. This would also hold true for greenhouse crops. Plant something instead of tomatoes where infection occurred the previous year.

3. Control the white fly as it is a serious pest, even if it carries no fungus.

4. Spray measures might be used as a means of control.

p.49

SUMMARY

1. Tomato anthracnoses are largely diseases of the fruit. Gloeosporium phomoides has been found to attack blossoms and young seedlings.

2. The causal organisms are Gloeosporium phomoides Sacc., Colletotrichum lycopersici Ches., and Melanconium sp. The main difference between Gloeosporium and Colletotrichum is the absence of setae in the former. The types of disease produced by the two are practically the same. Melanconium and

the type of anthracnose produced by it can be easily distinguished from the other types of tomato anthracnose by the black spore masses.

3. Tomato anthracnose is widely distributed throughout the United States and has been reported in Europe. The disease is of minor importance economically, but has been reported as increasing in economic importance in Indiana.

4. The disease is characterized by sunken areas on the fruit which are brown to black and dotted with fruiting pustules.

5. Gloeosporium presents different characteristics of growth and fruiting on different culture media.

6. The range of temperature for spore germination and growth of Gloeosporium lies between 4° and 37°C., the optimum being about 27°C.

7. These fungi enter the fruits mainly through wounds or other openings in the skin. They are both inter and intracellular in the tissues.

8. Gloeosporium is carried on seeds from diseased p.50 fruits. It may be disseminated by other agencies. Melanconium may be disseminated by white flies. This fungus has been found in the greenhouse only and would not be carried far by wind and water under these conditions.

9. The anthracnose diseases may be controlled by, (a) sanitation, (b) rotation of crops, (c) spraying (doubtful), (d) cold storage, (e) seed treatment, and (f) resistant varieties (possibly).

LITERATURE CITED

1. Arthur, J. C. Rot in ripe tomatoes. N.Y. Geneva Agr. Expt. Sta. Ann. Rept. 3: 380-382. 1884.

2. Chester, F. D. Anthracnose of the tomato. Del. Col. Agr. Expt. Sta. Ann. Rept. 4: 60-62. 1891.

3. __________ Anthracnose of the tomato. Del. Col. Agr. Expt. Sta. Ann. Rept. 5: 80. 1892.

4. __________ The ripe rot or anthracnose of the tomato. Del. Col. Agr. Expt. Sta. Ann. Rept. 6: 111-115. 1893.

5. Cobb, N. A. Pimply rot of the tomato. Agr. Gaz. N. S. Wales 6: 858-859. 1895.

6. Cook, M. T. and Taubenhaus, J. J. The relation of parasitic fungi to the contents of the cells of the host plants. Del. Col. Agr. Expt. Sta. Bul. 97 : 46. 1912.

7. Edgerton, C. W. The physiology and development of some anthracnoses. Bot. Gaz. 45: 403. 1908.

8. ______________ and Moreland, C. C. Diseases of the tomato in Louisiana. La. Agr. Expt. Sta. Bul. 142:21. 1913.

9. Groth, B. H. Alfred. Structure of tomato skins. N. J. Agr. Expt. Sta. Bul. 228. 1910.

10. Gueguen, M. F. Recerches anatomiques et biologiques sur la Gloeosporium phomoides Sacc., parasite de la tomate. Soc. Myc. France 18: 312-327.

p.51 11. Halsted, B. D. Experiments with tomatoes. N. J. St. Agr. Expt. Sta. Ann. Rept. 16: 293-296. 1895.

12. ______________ Experiments with tomatoes. N. J. St. Agr. Expt. Sta. Ann. Rept. 17: 333-336. 1896.

13. ______________ Tomato fruit rot. N. J. Agr. Col. Expt. Sta. Rept. of the Bot. Dept. 1903: 546-547.

14. Pool, V. W. "Ripe rot" or anthracnose of the tomato. Neb. Agr. Expt. Sta. Ann. Rept. 21: 9-15. 1908.

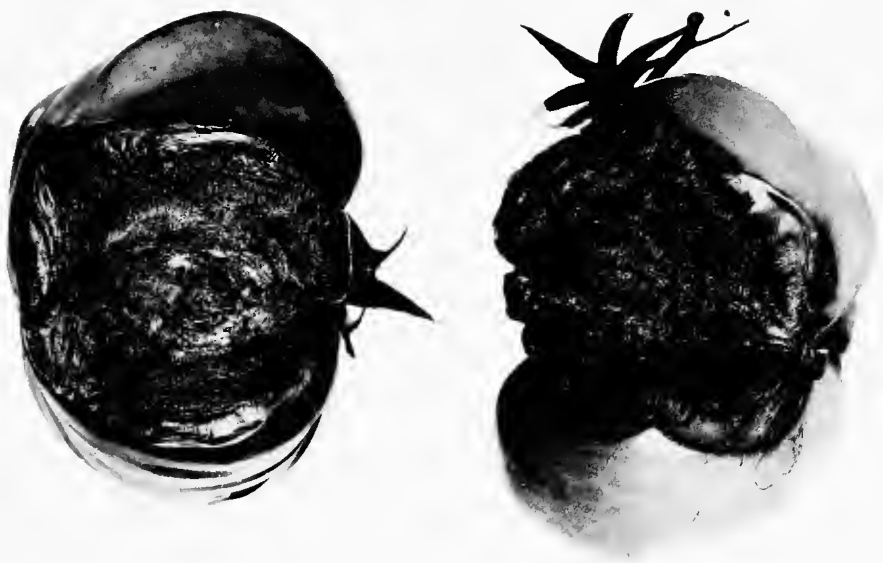
15. Rolfs, F. M. Tomato anthracnose. Fla. Agr. Expt. Sta. Ann. Rept. 1905: 45-46.

16. Saccardo. Gloeosporium phomoides. Syl. Fung. 3:718. 1884.

17. Stoneman, Bertha. Gloeosporium phomoides Sacc., on tomato. Bot. Gaz. 26: 74-76. 1898.

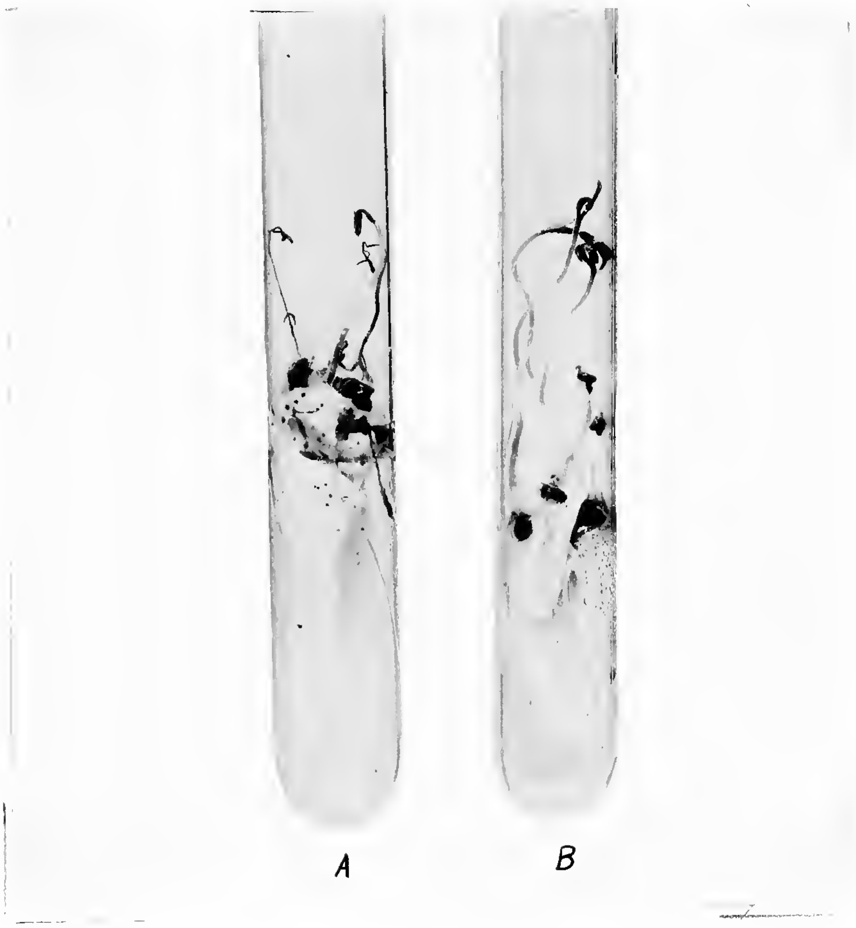
18. ______________ Colletotrichum lycopersici Ches. Bot. Gaz. 26: 95-96. 1898.

PLATE I



Anthracnose produced by *Gloeosporium phomoides* Sacc. on green tomato fruits. Inoculated artificially by needle prick.

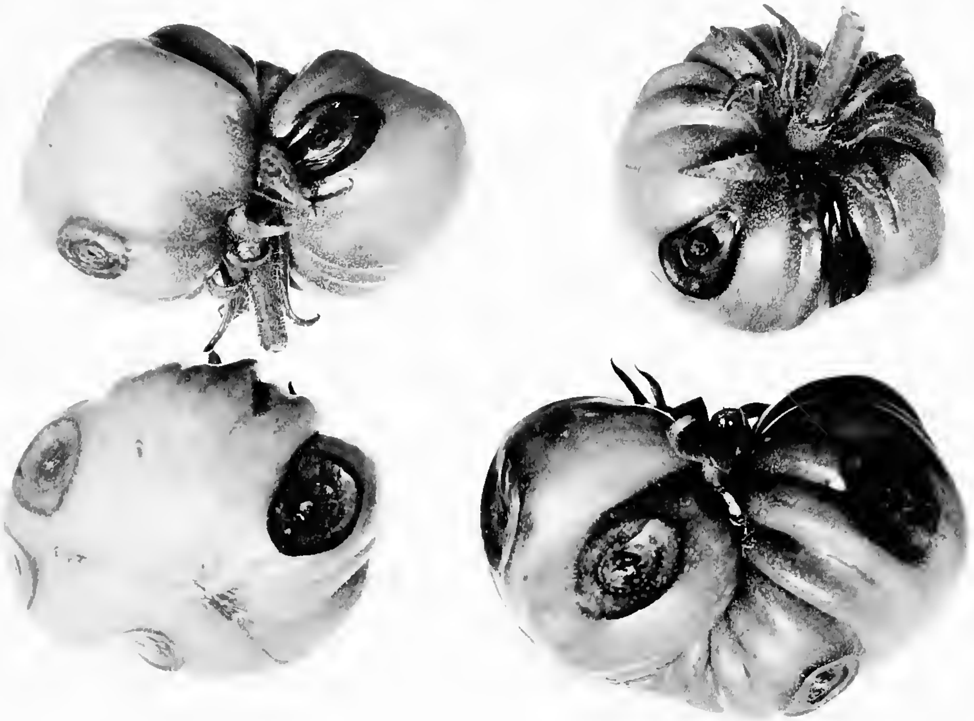
PLATE II



A. Seed from infected fruit kept dry five months and germinated on the sterile filter paper without treatment to kill the fungus. Notice the black perithecial bodies of Gloeosporium phomoides, and the death of the seedlings which the fungus has caused.

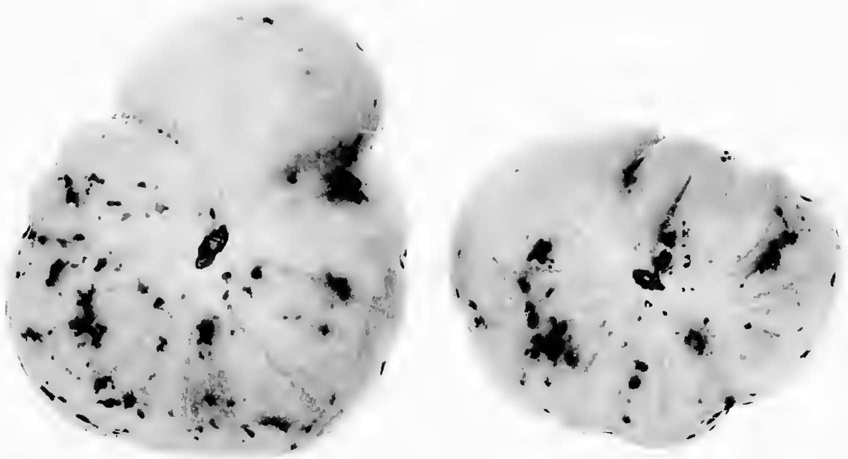
B. Seeds from the same source treated three minutes with $HgCl_2$ solution (1-1000). These seedlings remained free from the organism.

PLATE III



Melanconium sp. as found occurring naturally on tomato fruits. In this case the fruits were still green.

PLATE IV



Anthracnose of tomato fruits produced by artificial (needle prick) inoculations with Melanconium sp. The photograph was made nine days after inoculation.

Approved:

L. R. Jones

Sept. 20, 1915.